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THE DETECTION OF PHYTOSTEROL IN MIXTURES OF
ANIMAL AND VEGETABLE FATSBy ROBERT H. KERR,
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The testing of animal fats for adulteration with vegetable oils forms an important part of the work of the laboratory inspection of meat food products. In making such tests, while the physical and chemical constants may be of value in aiding the final decision, we are forced in the main to depend upon color reactions yielded by certain oils, and upon the presence of phytosterol in the suspected mixture. The color reactions, although extremely delicate, may lead to entirely erroneous conclusions. This is particularly true of the Becchi and Halphen tests for cottonseed oil. It is well known that the fat rendered from carcasses of animals which have been fed on cottonseed products responds to both the Becchi and Halphen tests. The feeding of cottonseed products to animals, particularly hogs, has become so common in the United States that we can no longer regard the Becchi and Halphen tests as positive indications of the presence of cottonseed oil, but rather as merely indications that cottonseed oil may be present. On the other hand, a negative result with these tests does not necessarily indicate the absence of cottonseed oil, for we know that by certain methods of treatment, particularly heating to a high temperature, the property of cottonseed oil to give these reactions is destroyed. As the fat of animals fed on cottonseed products does not contain phytosterol, it would seem that the presence of vegetable oils in fats can be proved with absolute certainty only by the detection of phytosterol in any given suspected mixture.

Methods for the detection of phytosterol are described in all of the important textbooks, and when the laboratory inspection under the meat-inspection law of 1906 was begun, the directions laid down by Lewkowitsch¹ were followed with satisfactory results. The principal objection to that method lay in the considerable time required for the test. As a result of efforts to shorten the method certain modifications of the details have been introduced which resulted in the saving of considerable time and also in avoiding troublesome steps in the process. The principal changes and advantages secured are (1) the employment of a larger sample, which gives a correspondingly larger amount of unsaponifiable material; (2) the extraction of the sample with alcohol, by which means the handling of large amounts of soap

¹ J. Lewkowitsch. *Chemical Technology and Analysis of Oils, Fats, and Waxes.* Third edition, p. 370. London, 1904.

is avoided; (3) the method of extracting the soap with ether, which avoids emulsions and allows the extraction to be accomplished in a few minutes; and (4) the treatment of the acetates, by means of which it is possible to obtain pure crystals from the second crystallization. The modified method has proved to be so satisfactory that it is given here for the benefit of others who may be engaged in a similar line of work.

DETAILS OF THE METHOD.

The method in detail is as follows:

SAMPLE.

The amount of sample used depends on the amount of material available. From 200 to 300 grams is the amount usually taken. The test is seldom attempted if less than 100 grams are available, and an amount greater than 500 grams is never taken.

EXTRACTION WITH ALCOHOL.¹

The sample is melted and poured into a flat-bottomed flask of 1-liter capacity which is closed with a rubber stopper perforated with three holes. This flask is set on the top of the steam bath and connected to a reflux condenser and to a 700 c. c. round-bottomed flask containing 500 c. c. of 95 per cent alcohol. A glass tube which is adjusted so that its lower end is about one-fourth of an inch above the surface of the fat and whose upper end is bent at a right angle and closed by means of a short piece of rubber tubing and a pinchcock fills the third hole in the stopper. The distilling flask is set down in the steam so that the alcohol boils briskly. The outlet tube reaches down to the bottom of the flask containing the sample so that the alcohol vapor as it distills over bubbles up through the fat and keeps it in a state of vigorous agitation. The alcohol vapor is condensed in the reflux condenser and returned to the flask containing the fat. The distillation is continued until all of the alcohol has collected in the flask containing the fat. The distilling flask is now disconnected. The alcohol in the flask immediately ceases to boil and soon separates from the fat. The empty distilling flask is next connected to the bent tube by a piece of glass tubing of sufficient length, the pinchcock opened, and the alcohol layer siphoned off into the distilling flask. This is then connected as before and the distillation continued until the alcohol has again collected in the first flask. It is then siphoned into the distilling flask as before, and a third extraction made. After the third extraction the alcohol layer is again siphoned off into the distilling flask and the fat is discarded. The alcohol now contains practically all of the cholesterol and phytosterol originally present in the fat.

SAPONIFICATION AND EXTRACTION WITH ETHER.²

The alcohol in the distilling flask is next concentrated by boiling to about 250 c. c., and 20 c. c. of a concentrated potassium-hydrate

¹ Method suggested by Mr. W. H. Low, of the Cudahy Packing Co.

² E. Salkowski. Über die Isolierung der Cholesterins aus den Fetten. Zeitschrift für Physiologische Chemie, vol. 57, no. 5/6, p. 515-528. Strassburg, Oct. 30, 1908.

solution (100 grams KOH dissolved in 100 c. c. water) added to the boiling liquid. It is boiled for 10 minutes to insure complete saponification of all the fat and is then removed from the steam bath and allowed to cool almost to room temperature. After it has cooled sufficiently it is poured into a large separatory funnel containing 500 c. c. of warm ether and shaken to insure thorough mixing. The mixture may be clear, but is more often opalescent. There is now poured in 500 c. c. of distilled water, and the funnel is rotated gently. Shaking must be avoided, as it leads to the formation of extremely stubborn emulsions, but the water should be mixed with the alcohol-ether-soap solution. Separation takes place at once and is clear and sharp. The soap solution is drawn off and the ether layer washed with 300 c. c. of distilled water, shaking being still avoided. After this washing it is washed repeatedly with small quantities of water until all soap is removed. The ether layer is then transferred to a flask and the ether distilled off. Distillation is stopped when the contents of the flask have been reduced to about 25 c. c., and the concentrated ether solution containing the cholesterol, phytosterol, and all other unsaponifiable matter is transferred to a tall 50 c. c. beaker. The evaporation is continued until all ether is driven off and the residue is perfectly dry. If desired, a tared beaker may be used and the weight of the unsaponifiable matter determined at this point.

PREPARATION OF THE ACETATES.

A small amount (3 to 5 c. c.) of acetic anhydrid is added to the dry residue in the beaker and heated to boiling over a free flame, the beaker being covered with a watch glass during the process. After a brief boiling—a few seconds is sufficient—the flame is removed and the beaker transferred to the steam bath and left there until the acetic anhydrid is driven off.

PURIFICATION OF THE ACETATES.¹

Thirty-five cubic centimeters of hot 80 per cent alcohol are added to the acetylated residue in the beaker and heated to boiling with vigorous stirring. The liquid is then filtered quickly through a folded filter and the insoluble residue washed well with boiling 80 per cent alcohol. The acetates of cholesterol and phytosterol are dissolved, while the greater portion of the impurities present are not dissolved by the alcohol and remain on the filter. Paraffin and paraffin oil, if present, are likewise separated by this treatment. The

¹ Mathes and Heintz discovered that the unsaponifiable portion of cottonseed oil contains a considerable amount of a body of doubtful character melting at 80° to 81° C., soluble in absolute alcohol but insoluble in 80 per cent alcohol and not affected by treatment with acetic anhydrid. Their observations have been confirmed in this laboratory. The method of purifying the acetates by treatment with 80 per cent alcohol is based on their work. (H. Mathes and W. Heintz. Über Baumwollsamenöl insbesondere die unverseifbaren Bestandteile. Archiv der Pharmazie, vol. 247, No. 3, p. 161-175. Berlin, June 19, 1909.)

combined filtrate and washings are next cooled to a temperature of 10° to 12° C. and allowed to stand at that temperature for two to three hours. During this time the acetates of cholesterol and phytosterol crystallize from the solution. They are removed by filtration, washed with cold 80 per cent alcohol, and then dissolved on the filter with a stream of hot absolute alcohol from a wash bottle, as little alcohol as possible being used. The alcoholic solution of the acetates is caught in a small glass evaporating dish, two or three drops of distilled water being added to the solution and heat applied if it is not perfectly clear. The dish is then set out on a desk in the laboratory and the alcohol allowed to evaporate spontaneously. The contents are stirred occasionally and the deposit of crystals which forms around the edges of the liquid and on the sides of the dish rubbed down into the solution with the stirring rod. As soon as a good deposit of crystals has formed they are removed by filtering through a hardened filter, washed twice with cold 90 per cent alcohol, and dried by suction. After drying by suction they are dried at 100° C. for half an hour and the melting point determined.

DETERMINATION OF THE MELTING POINT.

A tube of about 1 mm. diameter, sealed at one end and having a slight flare at the other, is filled to a depth of about 5 mm. with the dried crystals, which are packed somewhat firmly in the lower end by tapping on a hard surface. This is attached to the bulb of a suitable thermometer and the melting point determined. A thermometer graduated from 95° to 200° C. in one-fifth degrees is used in this laboratory. The determination is made in an Anschutz apparatus, the outer bulb being filled with concentrated sulphuric acid and the inner tube with glycerin. The apparatus is so adjusted that no correction of the observed temperature is required. The melting point of the first crop of crystals usually gives definite information as to the presence or absence of phytosterol, but the conclusion indicated is confirmed by recrystallizing from absolute alcohol and again determining the melting point. If the crystals are pure cholesterol acetate, the melting point of the second crop should agree closely with that of the first. If phytosterol acetate is present, however, a higher melting point should be noted, as phytosterol acetate is less soluble than cholesterol acetate.

Approved:

JAMES WILSON,

Secretary of Agriculture.

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